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RESULTS OF BIOLOGICAL STUDIES PERFORMED ABOARD THE "ZOND-5, 6, 7" STATIONS

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BIOASTRONAUTICS I

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ABSTRACT: Biological study of the earth-moon-earth trajectory was begun with an experiment on the "Zond-5" station (1968) and was continued with the flights of "Zond-6" and "Zond-7" stations. These stations carried tortoises, Drosophila, Tradescantia, common onion bulbs, dry seeds of wheat, barley, etc., different strains of Chlorella, enteric bacilli, and other objects. Physiological, morphological, histochemical, genetic, and other methods were used to study possible effects. In these experiments the research objects and methods were consistent with research on the "Vostok," "Voskhod," and "Kosmos" ships. The total dose of cosmic radiation was approximately the same on all three flights (3.5 rad). The main contribution to this dose was from penetrating radiation of the radiation belts received during fly-through. Details are given for each biological object used in the experiment. It was found that flight conditions on these three vehicles did cause definite shifts in the physiological functions and hereditary structures of some objects but both qualitatively and quantitatively these changes did not suffer from the shifts observed in experiments made in orbits below the radiation belts. Accordingly, radiation conditions on the investigated earth-moon-earth trajectory are not dangerous for manned flight provided that solar activity is at a low level.

The experiments carried out on the automatic "Zond-5, 6, 7" stations /1* represent a further step in the implementation of biological research in space. Their basic objective was a study of the changes in structures and functions of biological objects under the influence of weightlessness, ionizing radiations (primarily the penetrating radiation from the earth's radiation belts), and other factors involved in flight along the earth-moon-earth trajectory.

Almost 13 years have elapsed since the first biological experiment in space aboard the artificial earth satellite which carried the dog Layka (1957). During that period, Soviet and American researchers have accumulated

* Numbers in the margin indicate pagination in the foreign text.

a relatively great amount of information characterizing the biological (genetic) effect of spaceflight factors in orbits lying below the earth's radiation belts or penetrating slightly within them. The results have been sufficiently well generalized and analyzed in a number of thorough papers [1-5], and have already been applied in formulating appropriate recommendations concerning manned flight.

The biological study of the earth-moon-earth trajectory was begun with an experiment on the "Zond-5" station (1968) and was continued with the flights of the "Zond-6" and "Zond-7" stations.

Before proceeding to an exposition and analysis of some of the basic results, we will make some general comments of a methodological nature.

The stations carried tortoises, Drosophila, Tradescantia [spiderwort], /2
bulbs of Allium cepa [common onion], dry seeds of wheat, barley, etc., different strains of Chlorella, enteric bacilli, and other objects. Physiological, morphological, histochemical, genetic, and other methods were used in studying possible shifts. It should be noted that in these experiments there was consistency with respect to the objects and research methods with the data obtained on the "Vostok," "Voskhod" and "Kosmos" ships.

With respect to scope of research and range of biological objects, the experiments on the "Zond-5" and "Zond-7" stations were virtually identical; the experiment on "Zond-6" was made with an abbreviated program.

The material arrived at the laboratory at different times subsequent to flight termination. The experiment aboard the "Zond-5" station was least favorable in this respect.

The total dose of cosmic radiation registered by different types of dosimeters (direct-reading, thermoluminescent glasses, nuclear emulsions, etc.), placed at the sites of biological capsule attachment, during all three flights was approximately identical, about 3.5 rad; this corresponds to the computed data in [7]. The main contribution to the dose was from the penetrating radiation of the radiation belts during the time of fly-through. About 10% of the dose consisted of radiation received by the biological objects from a source present aboard the station.

Research on tortoises /3

a) Results of experiment on "Zond-5" station

As already reported [6, 8], the objects in this experiment were eight sexually mature male steppe tortoises (Testudo Horsfieldi Gray) weighing 340 to 400 g. Two animals (experimental group) were aboard the automatic station and two (control groups) were transported to the launching site and back; four (intact group) were kept in a vivarium. The tortoises in the experimental and control groups were starved and received no water for 39 days.

With respect to external appearance and behavior at the time of the postflight examination, the tortoises in all three groups exhibited no differences.

The alimentary activity of both the experimental and control animals, which had been flown to the launching site, and the intact animals, kept in the vivarium, was identically high. The weight of the tortoises carried aboard the station was low in comparison with the initial preflight weight by approximately 10%; in the control group, the weight loss during this period was 5%. No significant hematological shifts and electrocardiogram changes were exhibited by the examined animals.

A comparative microscopic study of organs from the experimental, control and intact tortoises, made by N. A. Gaydamakin, et al [8], revealed, in particular, the following changes. In the tortoises of the experimental and control groups, in comparison with the animals kept in the vivarium, the diameter of the intestine, thickness of the muscle wall, and length of mucous membrane villi had decreased. The epithelium of crypts exhibited cells with pyknotic nuclei and lipofuchsin inclusions. The mitotic activity of crypt /4
epithelium was depressed and the RNA content in epithelial cells was reduced. In the liver, the lipids were totally depleted, the volume of hepatocytes and the size of their nuclei were reduced, and the number of sperm cells in the seminal vesicles was smaller. The activity of succinate dehydrogenase and (to a lesser degree) alkaline phosphatase clearly increased in some elements of the intestinal wall, in the testes, liver, and kidneys. In these same organs, there was a decrease in the activity of monoaminoxidase and

α-glycerophosphate dehydrogenase.

b) Results of experiment on "Zond-7" station

The experiment was made on 30 sexually mature male steppe tortoises of the same species and approximately the same weight as on the "Zond-5" station. The vehicle carried four tortoises (experimental group); there were five control groups, one of which (four tortoises) made the trip to the launching site and back. The animals in the other four groups remained in the laboratory and were exposed to ionizing radiation, transverse accelerations and a combination of these two factors with parameters close to the in-flight factors. The tortoises in the experimental and control groups received no food or water for 27 days. The sixth group was a biological control; the animals were on an ordinary vivarium diet and were not exposed to any extremal factors. The experimental tortoises arrived at the laboratory on the fifth day after the vehicle landed.

It was found that the weight of all the animals, other than the biological control, decreased by 8.7 ± 0.7%. The tortoises kept in the vivarium on the ordinary diet gained during this time by an average of 10% of their initial weight. Thus, the difference was about 20%, taking into account the weight loss of the starving tortoises and the weight gain for the animals in the biological control. /5

The spaceflight conditions and transport to the launching site and back exerted no significant effect on the investigated peripheral blood indices (total number of leukocytes, erythrocytes, etc.) and the electrocardiogram for the animals.

According to data published by N. A. Gaydamakin, et al, in [9], in sections of the liver, kidneys, intestine, and testes, the activity of succinate dehydrogenase, monoaminooxidase, NAD-diaphorase, alkaline and acid phosphatases, as well as the content of fat, glycogen and RNA, were characterized by considerable individual variations. Unambiguous shifts in the determined indices could not be detected. The same applies to the results of cytological and histochemical investigations of the spleen, in whose sections the size and number of follicles, number of mitoses in the follicles, and number of active (pyroninphilic) reticular cells were determined. Some unidirectional data were obtained in the histological, karyometric (L. S. Sutulov, et al), and neurohistological (S. G. Kul'kin, et al) investigations of these animals. However, the detected changes were nonspecific, ordinary for the reaction of the tortoise body, and were also observed in the animals in the control groups exposed to the influence of some flight factors in the laboratory.

In analyzing the collected material, we noted the following important points.

In the tortoises which made the flight on "Zond-5" station the changes were for the most part of an atrophic nature. Definite differences were established in the degree of atrophic changes and histochemical shifts in the organs of the experimental animals and in the controls which had been flown to /6 the launching site and back. In the experimental tortoises, the atrophic changes were more clearly expressed; this can be attributed to the additional effect of spaceflight factors. This was indicated, in particular, by a decrease (in comparison with the control tortoises) in the organs of the experimental animals of the activity of monoaminooxidase, involved in the intimate metabolism of serotinin, whose level, according to some authors [10, 11], can change under the influence of flight conditions and individual extremal factors. However, if one takes into account the distinctive ecology of this species of animal, the marked seasonal variations in physiological activity and the intensity of metabolism in the tissues, dependent to a considerable degree on ambient temperature, it could also be postulated that these differences were caused to a certain degree by the presence of these animals in a tropical climate. A certain role could also be played by the conditions of their transport by sea from the site of launching. Comparison of the data obtained in this experiment shows that the principal structural shifts in the tortoises were caused by starvation and to a lesser degree, by exposure to flight conditions.

In the animals exposed aboard the "Zond-7" station, the organs also exhibited atrophic changes, but less clearly expressed than during flight aboard the "Zond-5" station. This is obviously attributable primarily to the fact that the starvation during the "Zond-5" experiment was more prolonged and the animals during some of the time were also exposed to a tropical climate.

An important peculiarity for the tortoises carried aboard the "Zond-7" station was the considerable individual variations in the degree of histochemical changes.

In conclusion, it should be noted that the changes detected in the tortoises which flew the earth-moon-earth trajectory are of an ordinary adaptive nature and can arise under the influence of a number of factors simulated in the laboratory. There were no detectable specific changes which could be attributed to exposure to weightlessness or the heavy component of cosmic radiation in these experiments. /7

Research on Drosophila

Several hundred eggs of Drosophila of the normal line Domodedova-32 were carried aboard the "Zond-5" station in the population chamber. By launching time they were to have reached the imago stage. An analysis was made of the second generation of individuals which upon return to the laboratory were in the late pupa and early imago stages. A study was made of the sex chromosomes of males and females and chromosome 2 for the purpose of determining the frequency of recessive lethals. Sublethals in the sex chromosome were also taken into account. Table 1 gives summarized data for this experiment.

TABLE 1.

Sex	Number of checked chromosomes 1	Lethals	
		n	% ± m
♂	2084	I2	0,57 ± 0,I6
♀	2000	2	0,I0 ± 0,07

Commas represent decimal points.

The increase in the frequency of lethals in the male sex chromosome in comparison with the spontaneous frequency, although not great, was nearly statistically reliable. The frequency of lethals in females in the experiment /8 and in the laboratory and in the laboratory control was approximately the same. This can evidently be attributed to the fact that the females received half of their X-chromosomes from F_1, in which no lethals could be present. It is also possible that the peculiarities of gametogenesis in the females and males, caused by differential elimination of lethal chromosomes, was also important. The frequency of autosomal lethals was also higher than in the experimental group, but this test was made on autosomes which were not checked out in advance and therefore, the results are of limited value [6].

Research on plants

The experiments were made with air-dried seeds of wheat, barley, peas, pine, carrot, tomato, and mustard, bulbs of the common onion, and the flowering plant Tradescantia paludosa. The seeds were exposed on all three stations, the bulbs on "Zond-5" and "Zond-7," and the plant on "Zond-5."

Evaluation of the biological effects of flight conditions was based on such criteria as seed germination, growth rate of sprouts, percentage of chromosomal rearrangements at the points of growth of primary roots, etc. Some of the seeds were left for field sowing.

Analysis of the investigations made by N. L. Delone, et al [11-13] revealed the following. The germination of the experimental and control seeds differed insignificantly, not counting the "growing old" wheat and pea seeds from the harvest of 1965. However, this fact is not new, since the phenomenon of "rejuvenation" of seeds which had lost their germinating force had already been observed during other flights [14-16].

As already mentioned, some of the wheat and barley seeds were planted in a field after flight on all three stations. It was discovered in these investigations that there was a stimulation of growth and development under poor cultivation conditions (low temperature in winter and spring, frit flies in the barley fields, etc.). /9

In order to determine the role of the temperature factor in the observed stimulation effect, N. L. Delone, et al carried out the following laboratory experiment. Some of the seeds which had been carried on "Zond-7" were divided into three groups. Seeds in the first group were cultivated at a temperature of +8°C, those in the second group - at +18 to 20°C, and those in the third group at +35°C. It was discovered that whereas in the second group there was no difference in the sprouting and rate of growth between the experimental and control plants, in the first and third groups the experimental plants were superior to the controls.

Chromosomal impairments were studied in all the flight experiments with dry seeds. It is interesting to note that during flights on the "Vostok," "Voskhod," and "Kosmos" ships in some cases there was a reliable increase in the percentage of chromosomal rearrangements. However, in these experiments, the sensitivity of the plants to spaceflight factors, determined from the number of chromosomal impairments, did not coincide with their sensitivity to γ-radiation. In experiments on the "Zond-5, 6, 7" stations, such a correlation was established for the first time. A reliable increase in chromosomal rearrangement was observed in the experimental barley and pine seeds, the seeds of the most radiosensitive plants among those participating in the experiment. This fact is illustrated in part by the data given in Table 2.

In an analysis of the data obtained in the "Zond-6" experiment, we note the discovery of special complex recombinations and the ejection of chromatid blocks into the cytoplasm. N. L. Delone, et al feel that these chromatid blocks are of the same nature as the "spherical fragments" which were registered /10
in the experiments with Tradescantia on the "Vostok" and "Voskhod" ships.

A study of the types of chromosomal impairments revealed that in the experimental material, it is chromosomal rearrangements which are most common, whereas, in the controls, chromatid rearrangements are most common. In the overwhelming majority of cases the number of recombinations predominates in the experiment, whereas, the control exhibits a predominance of fragments.

Interesting data were obtained by N. I. Nuzhdin, et al [18] in a cytological study of barley seeds which had flown on the "Zond-5" and

TABLE 2.
CHROMOSOMAL REARRANGEMENTS IN PRIMARY ROOTS OF SOME HIGHER PLANTS AFTER FLIGHT OF SEEDS AND COMMON ONION BULBS ON "ZOND-5"

Type of object	Groups	Number of anaphases	Number of rearrangements	% of rearrangements ± m	P
Wheat	Experiment	I128	3I	2,75 ± 0,49	0,34
	Control	I00I	30	3,00 ± 0,54	
Barley	Experiment	I74I	49	2,82 ± 0,40	3,43
	Control	798	8	I,00 ± 0,35	
Pine	Experiment	700	6I	8,7I ± I,06	5,22
	Control	II00	29	2,65 ± 0,48	
Pea	Experiment	I676	4I	2,45 ± 0,37	2,2
	Control	II08	I5	I,35 ± 0,35	
Bulb of common onion	Experiment	I085	2I	I,8 ± 0,4	2,7
	Control	2I2I	I7	0,8 ± 0,05	

Commas represent decimal points.

"Zond-6" stations. In these experiments, as in the studies by N. L. Delone, et al [12-17], it was demonstrated that spaceflight factors induce chromosomal mutations. With a combined exposure to spaceflight factors and γ-radiation, the effect is dependent on the physiological state of the material and the irradiation dose. Under some conditions, there is an additive effect of the two factors, whereas, in others irradiation exceeds the effectiveness of spaceflight factors.

Some results of these investigations are given in Table 3. This table shows reliable differences in the appearance of aberrant cells between the experimental and control variants in all series ($P < 0.02$ to < 0.001). Accordingly, spaceflight factors in both radioresistant (Pamir reproduction) and radiosensitive (Estonian reproduction) seeds induced an appearance of aberrant cells which was greater than in the control.

In the opinion of N. I. Nuzhdin, et al, the somewhat increased percentage of aberrant cells in the Pamir reproduction in comparison with the Estonian reproduction is caused by difference in the control and cannot be attributed to the influence of spaceflight factors. An increased percentage of cells with chromosomal impairments in the unirradiated material is a distinguishing characteristic of seeds of the Pamir reproduction in comparison with the Estonian reproduction. Accordingly, despite the great differences in the radiosensitivity of the seeds in the two reproductions, as can be judged from the percentage of aberrant cells in the control in series II and III, spaceflight factors induced an identical percentage of aberrant cells. Different results were obtained in series II and III, in which the seeds were irradiated before or after flight. In this case, spaceflight factors were considerably more effective in the Estonian reproduction. Accordingly, this is a variant in which the combined effect of γ-irradiation and spaceflight factors is more effective and is not manifested in a simple additive form.

The flight conditions on the "Zond-5" and "Zond-7" stations exerted a stimulating effect on the germination and growth of Allium cepa sprouts. The experimental groups exhibited an increase in the percentage of chromosomal rearrangements in comparison with the control ($R = 2.7$). /13

It should be noted that no chlorophylic or other mutations were discovered in the sown barley, wheat and onion.

The flowering plant Tradescantia was exposed on the "Zond-5" station. It was returned to the laboratory in a good condition, but unfortunately, at a late date. Analysis of chromosomal impairments in the rootlets revealed no substantial differences between the experiment and control. It is not impossible that the lack of rearrangements is attributable to the fact that the material was investigated too late, when there had been several generations of mitoses between the time when the material was fixed and the time when the

TABLE 3.

EFFECT OF SPACEFLIGHT FACTORS ON EMERGENCE OF ABERRANT CELLS IN ROOTLETS OF IRRADIATED AND UNIRRADIATED BARLEY SEEDS WITH DIFFERENT RADIOSENSITIVITY

Description of series	Pamir seed reproduction			Estonian seed reproduction		
	cells examined	aberrant cells	%	cells examined	aberrant cells	%
I. Unirradiated seeds						
Experiment	I54I	I00	6,4 ± 0,62	2455	II2	4,6 ± 0,43
Control	2548	III	4,3 ± 0,40	3889	I0I	2,6 ± 0,26
II. Seeds irradiated before flight in 10-krad dose						
Experiment	I594	200	I2,6 ± 0,82	I357	59I	43,5 ± I,30
Control	3473	353	I0,2 ± 0,50	2946	II27	38,2 ± 0,86
III. Seeds irradiated after flight in 10-krad dose						
Experiment	I392	20I	I4,3 ± 0,93	I366	600	43,9 ± I,3
Control	3162	384	I2,I ± 0,57	2945	II05	37,4 ± 0,89

Commas represent decimal points.

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flight ended [6].

Research on Chlorella and Lysogenic bacteria

The "Zond-5" station carried three strains of unicellular Chlorella algae: LARG-1, LARG-3, and LARG-5; the "Zond-7" station carried the strain LARG-1. A culture of lysogenic bacteria E. coli K-12 (λ) was used in experiments on both stations.

A study was made of the effect of spaceflight factors on the viability, Mutability, and dynamics of development of Chlorella cells. In analyzing the dynamics of cell sporulation, a count was made of the number of autospores forming during the first ("Zond-5"), and in the first and second sporulations ("Zond-7"). The basic results obtained by E. N. Vaulina, et al [19, 20] are summarized in Tables 4 to 6.

The experiment on "Zond-5" revealed a decrease in the viability of cells which had flown in space and some lag in their development (strains LARG-3 and LARG-5). The strains LARG-1 and LARG-5 exhibited an increase in the frequency of apparent mutations.

TABLE 4. /14

EFFECT OF SPACEFLIGHT FACTORS ON "ZOND-5" ON VIABILITY OF CHLORELLA CELLS

Strain	Variant	Viability %		
		% ± error	Control experiment ± m_{diff}	B
LARG-3	Control Experiment	94,6 ± 0,84 75,0 ± I,4I	I9,6 ± I,64	I2,0
LARG-5	Control Experiment	28,3 ± 2,08 3,6 ± 0,95	24,7 ± 2,28	I0,8
LARG-1	Control Experiment	9I,9 ± 0,97 85,I ± I,74	6,8 ± 2,0	3,4

Commas represent decimal points.

TABLE 5.

NUMBER OF MUTANTS OBSERVED IN DIFFERENT CHLORELLA STRAINS AFTER FLIGHT ON "ZOND-5"

Strain	Variant	Number of investigated colonies	Number of mutants in %		
			% ± error	Control experiment ± m_{diff}	t
LARG-3	Control	18891	0,875±0,068	0,070±0,109	0,64
	Experiment	12699	0,945±0,086		
LARG-5	Control	5292	0,340±0,080	0,990±0,130	7,6
	Experiment	12549	1,330±0,103		
LARG-1	Control	22842	0,495±0,046	0,815±0,146	5,6
	Experiment	6710	1,310±0,188		

Commas represent decimal points.

TABLE 6.

EFFECT OF SPACEFLIGHT FACTORS ON "ZOND-7" STATION ON CHLORELLA CELLS OF STRAIN LARG-1

Groups	Formation of autospores			Viability		Mutability	
	Number of examined cells	Sporulation nonequivalents % ± m	Cells in abnormal number of autospores % ± m	Number of examined cells	% ± m	Number of examined colonies	Number of mutants % ± m
Experimental	2543	I,30 ± 0,22	I,38 ±0,23	3804	98,75±0,24	57218	0,57±0,06
Control (launching site)	2452	I,43 ± 0,24	I,39± 0,24	3766	97,07±I,65	55000	0,66±0,04
Control (laboratory)	266I	I,32 ± 0,22	0,98± 0,I7	3907	98,98±I,33	59357	0,82±0,II

Commas represent decimal points.

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In the "Zond-7" experiment, there was a tendency to an increase in the percentage of viability and a decrease in the death of cells at different stages of development in comparison with the control. There was a slight tendency to an increase in the mean number of autospores and the antimutagenic effect of spaceflight factors (the difference between the experiment and the control was unreliable). /16

The combination of flight factors caused a moderate activation of prophagia in the lysogenic bacteria and exerted no effect on the frequency of formation of spontaneous auxotrophic mutations [21].

Thus, the cited data show that the flight conditions on the "Zond-5, 6, 7" stations caused definite shifts in the physiological functions and hereditary structures of a number of studied objects. It is extremely important that both qualitatively and quantitatively these changes in most cases did not differ from the shifts registered in experiments made in orbits below the radiation belts.

One exception is the results obtained in a number of experiments involving the cytogenetic investigation of pine and barley seeds ("Zond-5, 6, 7") and the cells of some Chlorella strains ("Zond-5"). The flight conditions on the stations caused a relatively large increase in the number of chromosomal rearrangements in pine and barley seeds and an increase in the number of mutants in the strains LARG-1 and LARG-5. At present, it is extremely difficult to tell what spaceflight factors were responsible for these impairments. Since this effect correlates with the radiosensitivity of these seeds and Chlorella strains, it can be surmised, for example, that it is /17
associated primarily with the influence of ionizing radiation of the radiation belts or the heavy component of primary cosmic rays. However, it is entirely obvious that both this and other possible hypotheses concerning the causes and mechanisms of the detected shifts require further checking and refinement.

The investigations made on the "Zond-7" station, as well as laboratory control experiments, made it possible to establish that some of the shifts detected in tortoises carried aboard the "Zond-5" station were caused primarily by factors unrelated to the flight.

Analysis of the collected data revealed that the radiation conditions on the investigated earth-moon-earth trajectory are not dangerous for manned flights provided that solar activity is at a low level.

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